nature genetics

Deletion of the late cornified envelope *LCE3B* and *LCE3C* genes as a susceptibility factor for psoriasis

Rafael de Cid[1,15,16], Eva Riveira-Munoz[1,16], Patrick L J M Zeeuwen[2,16], Jason Robarge[3,16], Wilson Liao[4], Emma N Dannhauser[5], Emiliano Giardina[6], Philip E Stuart[7], Rajan Nair[7], Cynthia Helms[3], Georgia Escaramís[1], Ester Ballana[1], Gemma Martín-Ezquerra[8], Martin den Heijer[9], Marijke Kamsteeg[2], Irma Joosten[10], Evan E Eichler[11], Conxi Lázaro[12], Ramón M Pujol[8], Lluís Armengol[1], Gonçalo Abecasis[13], James T Elder[7], Giuseppe Novelli[6], John A L Armour[5], Pui-Yan Kwok[4], Anne Bowcock[3], Joost Schalkwijk[2] & Xavier Estivill[1,14]

Psoriasis is a common inflammatory skin disease with a prevalence of 2–3% in individuals of European ancestry[1]. In a genome-wide search for copy number variants (CNV) using a sample pooling approach, we have identified a deletion comprising *LCE3B* and *LCE3C*, members of the late cornified envelope (LCE) gene cluster[2]. The absence of *LCE3B* and *LCE3C* (*LCE3C_LCE3B-del*) is significantly associated ($P = 1.38E{-}08$) with risk of psoriasis in 2,831 samples from Spain, The Netherlands, Italy and the United States, and in a family-based study ($P = 5.4E{-}04$). *LCE3C_LCE3B-del* is tagged by rs4112788 ($r^2 = 0.93$), which is also strongly associated with psoriasis ($P < 6.6E{-}09$). *LCE3C_LCE3B-del* shows epistatic effects with the *HLA-Cw6* allele on the development of psoriasis in Dutch samples and multiplicative effects in the other samples. *LCE* expression can be induced in normal epidermis by skin barrier disruption and is strongly expressed in psoriatic lesions, suggesting that compromised skin barrier function has a role in psoriasis susceptibility.

Psoriasis is a chronic hyperproliferative inflammatory disease of the skin, scalp, nails and joints that presents in several clinical forms[1]. Psoriasis is generally regarded as an immunologically mediated disorder characterized by abnormal keratinocyte proliferation and differentiation, local vascular changes and a mixed inflammatory infiltrate in the epidermis and dermis[3]. Psoriasis has a multifactorial etiology, involving environmental (infections, drugs, stress, smoking and climate) and genetic factors[4].

Genetic analyses of multiply affected families have found several susceptibility loci for psoriasis[5]. The most strongly associated is on chromosome 6p21 within the MHC region (*PSORS1*)[6]. Variants associated with the *HLA-Cw6* allele contribute between 33% and 50% of the familial clustering of the disease[7]. In addition to *PSORS1*, genome-wide linkage analyses and association studies have highlighted psoriasis loci on several other chromosomes[5,8], and advances in the identification of the relevant genes have mainly revealed molecules involved in the immune response and expressed in T cells and keratinocytes, such as HLA-C, IL12B, IL23R and β-defensins[9–11].

Copy number variation is a rich source of genetic variability[12]. Several copy number variants (CNVs) contribute to susceptibility to autoimmune and inflammatory disorders[13]. In a candidate gene approach, a higher copy number of the chromosome 8 β-defensin cluster has been demonstrated to be a susceptibility factor for psoriasis[11]. In order to identify genomic regions that vary in copy number in individuals with psoriasis, we conducted a genome-wide CNV analysis by array comparative genomic hybridization (aCGH) using the human Agilent 244A array, which contains approximately 244,000 probes and covers the entire genome with a 10-kb resolution. Here, we report the characterization and replication study of one such region on human chromosome 1q21, which was specifically characterized because it falls within a previously known psoriasis susceptibility locus (*PSORS4*)[14].

To reduce detection of interindividual CNV variability unrelated to psoriasis, we used a pooling approach. We assembled equimolar amounts of DNA in three pools, each containing 20 psoriasis samples.

[1]Genes and Disease Programme, Centre for Genomic Regulation (CRG) and Public Health and Epidemiology Network Biomedical Research Center (CIBERESP), 08003 Barcelona, Spain. [2]Department of Dermatology and Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, 6525 Nijmegen, The Netherlands. [3]Department of Genetics, Washington University School of Medicine, Saint Louis, Missouri 63110, USA. [4]University of California, San Francisco, San Francisco, California 94143, USA. [5]Institute of Genetics and School of Biology, University of Nottingham, Queen's Medical Centre, NG72RD Nottingham, UK. [6]Department of Biopathology, Tor Vergata University of Rome, 00133 Rome, Italy. [7]Department of Dermatology, University of Michigan, Michigan 48109, USA. [8]Dermatology Service, Hospital del Mar-IMAS, 08003 Barcelona, Spain. [9]Department of Endocrinology and Department of Epidemiology and Biostatistics and [10]Department of Bloodtransfusion and Transplantation Immunology, Radboud University Nijmegen Medical Centre, 6525 Nijmegen, The Netherlands. [11]Department of Genome Sciences, University of Washington, Seattle, Washington 98195, USA. [12]Catalan Institute of Cancer, L'Hospitalet de Llobregat, 08907 Barcelona, Spain. [13]Department of Biostatistics, School of Public Health, University of Michigan, Michigan 48109, USA. [14]Pompeu Fabra University (UPF), 08003 Barcelona, Spain. [15]Present address: Centre National de Génotypage, 91057 Evry, France. [16]These authors contributed equally to this work. Correspondence should be addressed to X.E. (xavier.estivill@crg.es).

Received 23 May 2008; accepted 22 December 2008; published online 25 January 2009; doi:10.1038/ng.313

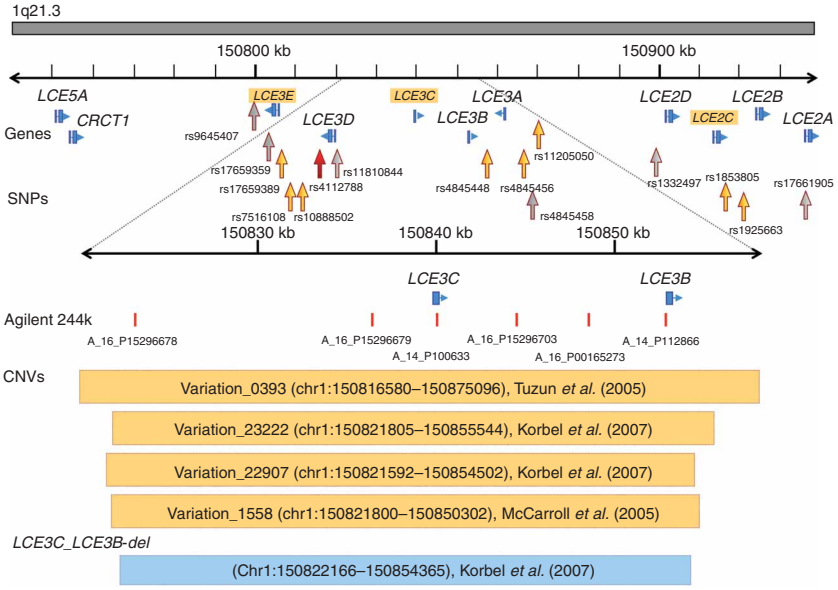

Figure 1 Region of the LCE cluster containing the *LCE3C_LCE3B-del* associated with psoriasis. The region shows 200 kb of the genomic region between *LCE5A* and *LCE2A* on human chromosome 1. The 11 genes of these regions are shown with their transcription orientation. Vertical yellow arrows indicate the position of the SNPs that showed association with psoriasis and with *LCE3C_LCE3B-del*. Vertical gray arrows indicate the position of those SNPs that did not show association with psoriasis. The location of the rs4112788 SNP is shown with a red arrow. The location of the Agilent probes that showed variability in aCGH experiments are shown as vertical bars. The CNV regions described in other studies are indicated. The *LCE3C_LCE3B-del* deletion, which spans 32,199 bp, is highlighted. The genes for which TaqMan assays were developed are shown on a yellow background.

Each of these three pools was analyzed against a pool of 50 samples from unrelated subjects of the general population in the aCGH experiments. Four regions fulfilled criteria for a CNV potentially associated with psoriasis (**Supplementary Methods** online) and all corresponded to previously identified CNVs (Database of Genome Variants; **Supplementary Table 1** online). One such region on chromosome 1q21 involved, at least in one pool, four contiguous probes with $\log_2$ ratios >0.3 and two additional probes with $\log_2$ ratios >0.25, with the six probes spanning the *LCE3C* and *LCE3B* genes, members of the LCE cluster[2] (**Fig. 1**).

To evaluate whether the individual samples of subjects with psoriasis had a reduction in copy number of *LCE3C* and/or *LCE3B* genes with respect to controls, as predicted by the aCGH study, we carried out quantitative PCR (qPCR) on 557 Spanish samples, including those used in the aCGH experiments (**Supplementary Table 2** online). We targeted qPCR to exonic regions of *LCE3C*, and two flanking genes, *LCE2C* and *LCE3E*. We found that this CNV was biallelic and that 33% of the DNA samples from subjects with psoriasis had a reduction in the *LCE3C* copy number, compared with 24% of control samples (OR = 1.62; 95% CI = 1.04–2.51, $P = 0.02$).

The LCE region has previously been defined in several studies as being a CNV[12] (**Fig. 1**), but only recently has it been characterized as a 32.2-kb deletion (from nucleotides 150,822,166 to 150,854,365 in hg18)[15,16]. We took advantage of these CNV coordinates to define the deletion breakpoints and develop a direct assay of the *LCE3C* and *LCE3B* deletion *(LCE3C_LCE3B-del)* by PCR amplification. We found a significantly higher frequency of the *LCE3C_LCE3B-del* allele in individuals with psoriasis (64%) compared with controls (55%) ($P = 0.0028$) (**Table 1**). Concordance of results between *LCE3C* qPCR and direct PCR typing of *LCE3C_LCE3B-del* was excellent (weighted kappa = 0.93; 95% CI = 0.88–0.95).

To replicate the association of *LCE3C_LCE3B-del* with psoriasis initially found in the Spanish samples, the *LCE3C_LCE3B-del* frequency was assessed by PCR typing in four case-control samples from Italy ($n = 900$), the United States ($n = 890$) and The Netherlands ($n = 484$) (**Table 1**), and in a family-based study from the United States (1,395 affected in 562 families) (**Supplementary Table 2**). In all studies, we observed an increased risk for psoriasis in carriers of the *LCE3C_LCE3B-del* allele (OR = 1.30–1.50). Overall, the frequency of *LCE3C_LCE3B-del* was significantly higher in individuals with psoriasis (68%) than in control subjects (59%) ($P = 1.38E{-}08$), according to a logistic model in which population was introduced as a confounding variable after nonsignificant heterogeneity was detected by population (**Table 1**). A total of 334 University of Michigan samples were informative for *LCE3C_LCE3B-del* using a family-based association test (FBAT) leading to an association with psoriasis ($P = 5.4E{-}04$); (**Supplementary Table 3** online). The genotype analysis that estimated the OR for heterozygous and homozygous carriers suggests a potential dosage effect (**Supplementary Table 4** online). Thus, the presence of two copies of the *LCE3C* and *LCE3B* genes is protective against the

Table 1 Association of *LCE3C_LCE3B-del* with psoriasis in 2,831 subjects of European ancestry from different populations

Population (samples)	Phenotype	Alleles	*LCE3C_LCE3B-del* (%)	*LCE3C_LCE3B* (%)	OR (95% CI)	*P* value
Spanish (557)	Psoriasis	350	225 (64)	125 (36)	1.47 (1.28–1.72)	0.0028
	Control	764	420 (55)	344 (45)		
Italian replicate (900)	Psoriasis	900	573 (64)	327 (36)	1.30 (1.12–1.51)	0.006
	Control	900	516 (57)	384 (43)		
Dutch replicate (484)	Psoriasis	408	281 (69)	127 (31)	1.50 (1.29–1.74)	0.0033
	Control	560	334 (60)	226 (40)		
USA replicate (890)	Psoriasis	1,192	847 (71)	345 (29)	1.36 (1.18–1.58)	0.0038
	Control	588	378 (64)	210 (36)		
Total (2,831)	Psoriasis	2,852	1,926 (68)	924 (32)	1.38 (1.19–1.61)	1.38E–08
	Control	2,812	1,648 (59)	1,164 (41)		

Odds ratio (OR) and 95% confidence interval 95% (CI) for *LCE3C_LCE3B* deleted allele *(LCE3C_LCE3B-del)* and psoriasis were obtained by logistic regression, overall and separately for each of the four populations; Overall OR and *P* values are standardized by population according to a logistic model in which population was introduced as a confounding variable after (nonsignificant) heterogeneity was detected by population.

Table 2 Association of rs4112788 genotypes in the *LCE* cluster with psoriasis in Spanish, Italian, Dutch and US samples

			Recessive model C/C versus T/T and C/T		Dominant model C/C and C/T versus T/T		Log-additive model			
Population	Psoriasis[a]	Controls[a]	OR	95% CI	OR	95% CI	OR	95% CI	*P* value	r^2
By study										
Spanish	175	382	1.58	1.09–2.30	2.67	1.46–4.88	1.62	1.23–2.13	4.93E–04	0.92
Italian	449	446	1.35	1.03–1.77	1.54	1.06–2.24	1.30	1.08–1.58	0.0065	0.88
Dutch	202	278	1.50	1.04–2.17	2.08	1.17–3.70	1.47	1.12–1.93	0.0046	0.99
United States	587	293	1.46	1.10–1.94	1.54	0.93–2.55	1.38	1.10–1.72	0.0051	0.95
Overall	1,413	1,399	1.54	1.32–1.79	1.98	1.57–2.49	1.48	1.33–1.66	3.38E–12	
Overall adjusted	1,413	1,399	1.45	1.24–1.70	1.79	1.41–2.28	1.41	1.25–1.58	6.56E–09	

Odds ratio (OR) and 95% confidence interval (CI) for psoriasis comparing homozygous (C/C), heterozygous (C/T) and homozygous genotype (T/T) of rs411178 overall and separately for each of the four populations. Overall OR are standardized by population according to a logistic model in which population was introduced as a confounding variable after (nonsignificant) heterogeneity was detected by population. *P* values are derived from the log-additive model, with homozygotes for the C allele at higher risk for psoriasis than homozygotes for T/T as a basal genotype. Predictive performance of allele with deletion of *LCE3C* and *LCE3B* is presented for each population using the coefficient of determination measure (r^2).
[a]Subjects with valid call for rs4112788.

development of psoriasis ($P < 0.0001$). The percentage of psoriasis risk attributed to carrying at least one deletion-containing allele (estimated population attributable risk) was 21% in the combined dataset.

We evaluated linkage disequilibrium (LD) between *LCE3C_LCE3B-del* and tag SNPs covering the *LCE3* and *LCE2* region (**Fig. 1**, **Supplementary Table 5** and **Supplementary Fig. 1** online). We found 14 SNPs strongly associated with *LCE3C_LCE3B-del* (**Supplementary Table 6** online). The region of association with *LCE3C_LCE3B-del* spans over 130 kb, with allele C at rs4112788 ($P = 2.40E–126$) being in strongest LD ($r^2 = 0.93$ and $D' = 0.99$). rs4112788 maps 584 nucleotides downstream of *LCE3D* and 4.5 kb centromeric to *LCE3C_LCE3B-del*, and the strong LD suggests a single origin in the population of European ancestry. The analysis of rs4112788 in all populations showed that this SNP is strongly associated with psoriasis (overall adjusted *P* value < 6.6E–09; **Table 2**). rs4112788 is also associated with psoriasis in the US family sample by FBAT ($P = 0.0019$; **Supplementary Table 3**).

The region of association with psoriasis detected and investigated in our study is in the region of the *PSORS4* locus, originally identified in Italian families[14]. The *PSORS4* locus contains the epidermal differentiation complex (EDC), a cluster of at least 20 genes expressed during epithelial differentiation[2,17]. Genes in the *PSORS4* region have been investigated in several studies but none has been clearly shown to be involved in psoriasis[8,18]. However, the genes reported in those studies are in completely distinct LD blocks from *LCE3B* and *LCE3C*, showing a low LD with rs4112788 ($r^2 < 0.5$). A comprehensive dissection of this region of chromosome 1 could provide further clues about the involvement of other genes besides *LCE3B* and *LCE3C* that could explain previous linkage and association findings in psoriasis.

We evaluated the relationship between the *LCE3C_LCE3B-del* locus and the *HLA-Cw6* allele (as part of *PSORS1*)[7,9]. To test for an interaction between *PSORS1* and the *LCE3C_LCE3B-del* in the Spanish sample, we analyzed six SNPs in the region (**Supplementary Table 7** online). These SNPs are in strong LD with *HLA-Cw6* (ref. 19) and as expected, each of them was highly associated with psoriasis and defines a strongly associated haplotype in the region (OR = 6.62, 95% CI = 4.12–10.64, $P < 0.00001$, 10,000 permutations; **Supplementary Table 8** online). We did not find any evidence of interaction in either the Spanish and Italian case-control samples or the US family samples, but we did observe this for the Dutch sample, where the presence of the *LCE3C_LCE3B-del* allele (as well as rs4112788) confers a significant risk for psoriasis only in presence of *HLA-Cw6* (**Table 3**).

The identification of an epistatic effect between the *HLA-C* and *LCE3C* loci in the Dutch population is notable because attempts to identify interaction between *HLA-C* and other loci have failed[20]. Nevertheless, an epistatic effect between the *PSORS1* and *PSORS4* loci was reported in Italian families[21]. Further work will be needed to understand whether significant epistasis detected only in Dutch samples is observed because of sampling error, population-specific effects or other aspects of methodology.

Table 3 Genetic interaction analysis between *LCE3C_LCE3B-del and LCE3C_LCE3B-del* tag SNP and *PSORS1* loci

			rs4112788 (log-additive)		*LCE3C_LCE3B-del* (log-additive)		*HLA-Cw6* positive vs. negative		Epistasis[a]						
										rs4112788 – HLACw6			*LCE3C_LCE3B-del* –HLACw6		
	Psoriasis	Controls	OR	95% CI	OR	95% CI	OR	95% CI	*Cw6*	OR	95% CI	*P*	OR	95% CI	*P*
Spanish	175	382	1.61	1.23–2.13	1.63	1.23–2.15	2.56	1.68–3.92	+	1.86	1.34–2.57	0.213	1.87	1.34–2.60	0.247
									–	1.23	0.70–2.15		1.27	0.72–2.22	
Italian	450	450	1.30	1.08–1.59	1.28	1.06–1.54	2.50	1.86–3.36	+	1.25	0.87–1.81	0.875	1.14	0.80–1.62	0.544
									–	1.30	1.02–1.66		1.30	1.03–1.64	
Dutch	202	278	1.47	1.11–1.93	1.49	1.14–1.96	3.45	2.27–5.25	+	2.58	1.46–4.57	0.014	2.60	1.47–4.59	0.016
									–	1.15	0.83–1.59		1.17	0.84–1.63	

PSORS1 locus definition; in the Italian and Dutch samples direct HLA-Cw6 typing was available and was analyzed using the carrier status definition for *Cw6* allele. In the Spanish sample rs1062470 is used as a proxy for *HLA-Cw6* by a dominant model of heritability for allele T (which is referred here as the positive group).
[a]For the epistasis analysis, logistic regression models were carried out which included an interaction term, (rs4112788 – HLA-Cw6 or *LCE3C_LCE3B-del* –HLA-Cw6); *P* values were derived from the log-likelihood ratio test between the model including both additive effects plus the interaction term (rs4112788/ *LCE3C_LCE3B-del* –HLA-Cw6 + rs4112788 – HLA-Cw6) against the model that only includes additive effects. OR indicate association of rs4112788/ *LCE3C_LCE3B-del* with psoriasis stratified by positive and negative *HLA-Cw6* alleles. Plus sign (+), *HLACw6* positive; minus sign (–), *HLACw6* negative.

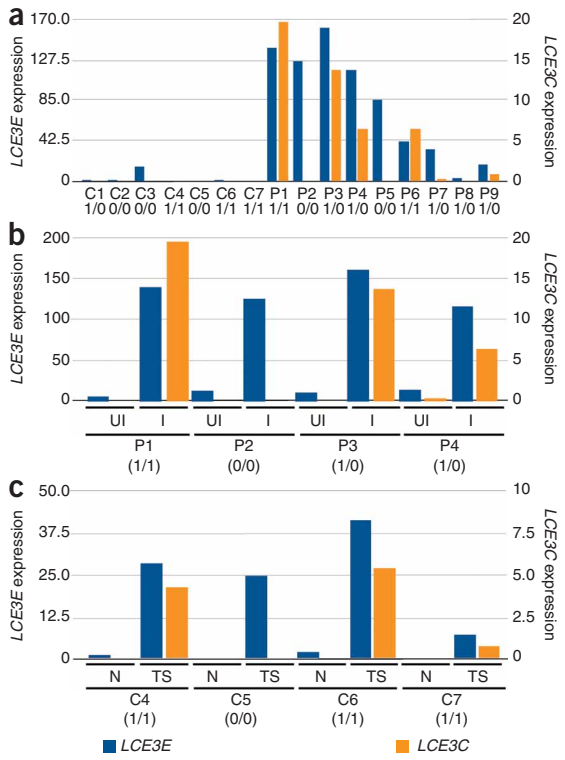

Figure 2 Patterns of mRNA expression of *LCE3C* in the epidermis of psoriatic and control subjects. (**a**) Evaluation of the expression profiles of *LCE3C* and *LCE3E* in the skin of control subjects (C1 to C7) and subjects with psoriasis (P1 to P9). (**b**) Evaluation of the expression of *LCE3C* and *LCE3E* in uninvolved (UI) skin versus involved lesional (I) skin of subjects with psoriasis. Expression levels (fold increase relative to the endogenous gene *RPLP0*) of *LCE3E* and *LCE3C* are represented by left and right *y*-axis values, respectively. The genotypes 0/0, 0/1 and 1/1 correspond to the *LCE3C_LCE3B-del* homozygotes, heterozygotes and homozygotes for the presence of *LCE3C* and *LCE3B*. (**c**) Induction of expression of *LCE3C* and *LCE3E* in normal skin (N) by the tape-stripping (TS) model, which removes the stratum corneum of the epidermis.

Finally, to further investigate the potential role of this CNV in psoriasis, we evaluated the patterns of expression of *LCE3C* in normal skin and in lesional and uninvolved psoriatic epidermis. qRT-PCR assays were designed for *LCE3C*, which is contained in the deletion, and for *LCE3E*, which is outside the deletion. qRT-PCR was done on purified epidermal cells from seven normal skin biopsies and from lesional epidermis of nine subjects with psoriasis (**Fig. 2a**). *LCE3C* and *LCE3E* expression was low to undetectable in all of the normal skin samples, irrespective of their *LCE3C_LCE3B-del* genotype, in agreement with what has been described previously[2]. Among subjects with psoriasis, only those harboring at least one copy of the *LCE3C* gene showed *LCE3C* expression. All samples from lesional skin showed expression of *LCE3E*. For psoriasis samples 1 to 4, mRNA from purified epidermal cells of uninvolved skin was also available. **Figure 2b** shows that, similar to skin of healthy controls, no *LCE3C* or *LCE3E* expression is detected in uninvolved psoriatic skin, irrespective of the *LCE3C_LCE3B-del* genotype. We hypothesized that skin barrier disruption might be a physiological stimulus for *LCE3* gene induction. We used tape stripping of normal skin as a model to investigate this in healthy volunteers. This procedure involves successive application of adhesive tape, which removes the stratum corneum, the top layer of the epidermis. The subsequent epidermal response involves keratinocyte hyperproliferation, accelerated differentiation and increased expression of genes involved in skin barrier formation. Although *LCE3C* and *LCE3E* mRNA was absent in normal skin, we found that their expression was markedly induced by tape stripping (**Fig. 2c**), except in the case of one control individual (C5) with two *LCE3C_LCE3B-del* alleles who did not show *LCE3C* mRNA induction, as expected. Expression of the nondeleted *LCE3E* gene was induced in all individuals following tape stripping.

In healthy individuals, the expression of induced *LCE3C* may vary according to the individual response to tape stripping. If we assume, however, that *LCE3C* and *LCE3E* are subject to similar regulation mechanisms, we can correct for these nongenomic effects on gene expression. To this end, we plotted the ratio of *LCE3C* to *LCE3E* expression for 17 individuals against the number of nondeleted allele copies of the *LCE3C* gene. A significant correlation was found (Pearson's $r = 0.88$, $P = 2.0\text{E}{-}06$) between the normalized *LCE3C* expression and copy number (**Supplementary Fig. 2** online).

This genome-wide analysis has led to the identification of a region of chromosome 1q21 that harbors two genes (*LCE3C* and *LCE3B*) that are deleted in a significant fraction of individuals of European ancestry with psoriasis. Several SNPs in the region are associated with the deletion, one of them (rs4112788) being a close proxy of *LCE3C_LCE3B-del*. This strong LD of SNPs with a given CNV might be a common feature of biallelic CNVs, particularly useful in association studies for complex disorders, as has recently been detected for Crohn's disease and the deletion polymorphism upstream of the *IRGM* gene[22]. This might not be the case for complex CNVs, such as the β-defensin cluster on chromosome 8p23, which shows a significant association between increased β-defensin gene copy number and psoriasis susceptibility[11]. The complex nature of the β-defensin CNV likely precluded its identification in the aCGH done here. Because of the strong LD between the CNV and rs4112788, it is not possible to define which variant better captures the association signal with psoriasis. However, lack of evolutionary conservation suggests that rs4112788 is a nonfunctional variant, not included in any promoter, enhancer or distant regulatory element (NCBI DCODE), making the deletion of the two *LCE* genes a more suitable candidate for the association detected here. However, both *LCE3C_LCE3B-del* and rs4112788 could be merely markers for another, unknown and untested variant. This will be resolved only after resequencing and fine mapping of the region and by the analysis of the potential joint contribution of other genes. We have also shown that *LCE3C* expression is induced upon epidermal activation, as found in psoriatic and induced skin lesions. As 30% of the general population is not able to express these genes as a result of the deletion, there is probably some redundancy in the function of *LCE* genes in the cluster. It is possible that these other genes assume the functions of *LCE3C* and *LCE3B* imperfectly, contributing to the abnormal differentiation and epidermal hyperproliferation characteristic of psoriatic lesions. We would speculate that absence of intact *LCE3C* and *LCE3B* genes could lead to an inappropriate repair response following barrier disruption, which is insufficiently backed up by other *LCE* genes. A more leaky epidermal barrier following skin injury would allow easier penetration of exogenous agents (allergens and/or microorganisms)[23], which against a genetic background of HLA-Cw6 positivity could evoke a response of the adaptive immune system leading to overt inflammation.

METHODS

Subjects. We obtained ethical approval by the relevant research ethics committees of each center for collection of all DNA samples and written informed consent from all participants (from Spain, The Netherlands, Italy and the United

States). All subjects were of European descent. For details on diagnostic criteria and other characteristics of the subjects, see **Supplementary Note** online.

Identification of CNV regions. To investigate the presence of CNVs associated with psoriasis, we carried out a DNA-pooling approach for aCGH using the Human Genome CGH Microarray Kit 244A, which contains over 244,000 probes and covers the entire genome with a 10-kb resolution between probes. Regions composed of at least three altered probes (including the entry probe) were retained for further study. Results observed in the 1q21 region for aCGH analysis were confirmed by a second technique using a qPCR analysis. For details on CNV analysis and results and characterization in each population sample see **Supplementary Methods**.

SNP genotyping. SNPs were genotyped using different platforms. We tested each polymorphism in the whole group to ensure consistency with Hardy-Weinberg equilibrium (HWE). Because of multiple testing, we used a threshold of $P = 0.001$. The final SNP dataset consisted of 15 SNPs (**Supplementary Table 5**) with an overall genotype call rate of 99%. See **Supplementary Methods** for details on SNP genotyping methods in each population sample. Primer sequences are available in **Supplementary Table 9** online.

***LCE3C_LCE3B –del* and SNP association analysis.** Logistic regression models were used to assess the genetic effect of the *LCE3C_LCE3B –del* and SNPs on psoriasis risk. Intergroup comparisons of genotype frequency differences were done by regression analysis for co-dominant and genotype-specific models. Unadjusted crude odds ratios (OR) and 95% confidence intervals (95% CI) were calculated. Analysis was carried out using the SNPassoc R library, from The Comprehensive R Archive Network. For SNP association analysis, we used Bonferroni-corrected values to account for the problem of multiple testing (accounting for multiple testing of 15 polymorphic SNPs and the genotype-specific models tested[24]) to a threshold P value of 0.0015.

Interaction analysis. For the interaction analysis in the Spanish population, we used the genotype-specific model of heritability for each SNP based on the Akaike criteria (AIC) derived from the association analysis. We derived the P values for the interaction (epistasis) using the log-likelihood ratio test comparing the full interaction model to the additive SNP model, and the multiplicative effect using the likelihood ratio test of the additive model with the likelihood of the best model using single SNPs (**Supplementary Fig. 3** online; codominant models). A P value of 0.00054 for significance after Bonferroni correction to account for multiple comparisons was used. For the Italian and Dutch samples, interaction analysis *of LCE3C_LCE3B-del* or rs4112788 and *HLA-Cw6* was done. Assessment for significant epistasis and multiplicative effects was carried out as described for the Spanish population. For analysis of interaction in the Michigan family sample (**Supplementary Tables 10** and **11** online), we followed a previously described method[25].

Biopsies, RNA isolation and quantitative real-time PCR. All individuals with psoriasis had plaque-type psoriasis diagnosed by a dermatologist. Biopsies of normal, psoriatic and tape-stripped skin were taken under local anaesthesia. Tape stripping of human skin is the repeated application and removal of adhesive tape, which produces a standardized injury of the epidermis by removal of the stratum corneum[26]. All controls and cases were of native European Dutch origin. Permission for these studies was obtained from the local medical ethics committee ("Commissie Mensgebonden Onderzoek Arnhem-Nijmegen"), and volunteers gave written informed consent. The study was conducted according to the Declaration of Helsinki ethical principles. Detailed procedures for preparation of purified epidermis, RNA isolation, and real-time quantitative PCR are given as **Supplementary Methods**.

Note: Supplementary information is available on the Nature Genetics website.

ACKNOWLEDGMENTS

We gratefully acknowledge the participants in the genetic studies whose contributions made this work possible and personnel at the CeGen genotyping core facility (Barcelona node). We thank T. Henseler, S. Jenisch, M. Weichenthal and E. Christophers from the University of Kiel for making samples from Kiel, Germany available for analysis. We thank A. Poon for technical assistance, the University of California San Francisco Psoriasis Center Staff for help with subject recruitment and J. Gartlon for proofreading the manuscript. This work was supported in part by funds from the "Generalitat de Catalunya," the Spanish Ministry of Science and Innovation and the European Commission ENGAGE Project (X.E., R.d.C., L.A., E.R.-M., G.E. and E.B.), ADIPSO (G.N.) and National Institutes for Arthritis, Musculoskeletal and Skin Diseases (A.B., J.T.E., R.N. and P.E.S.). E.R.-M. is supported by CIBERESP (Carlos III Health Institute). J.T.E. is supported by the Ann Arbor Veterans Affairs Hospital. W.L. is supported by a grant from the Dermatology Foundation.

AUTHOR CONTRIBUTIONS

R.d.C., E.R.-M., P.L.J.M.Z. and J.R. contributed equally to this work. R.d.C., G.M.-E., R.M.P. and C.L. recruited the subjects from Spain. P.L.J.M.Z., M.K., M.d.H. and J.S. recruited the subjects from The Netherlands. E.G. and G.N. recruited the subjects from Italy. P.-Y.K., A.B., R.N., W.L. and J.T.E. recruited the subjects from the USA. R.d.C., E.R.-M., P.L.J.M.Z., J.R., W.L., E.N.D., E.G., I.J., C.H., E.B., P.E.S. and R.N. performed the genotyping and experimental work. R.d.C., L.A., G.E., G.A., P.E.S., J.S. and X.E. analyzed the data. J.S. was responsible for the design and execution of the expression studies in keratinocytes. X.E., G.N., J.T.E., E.E.E., J.A.L.A., P.K., A.B. and J.S. supervised the work. X.E. designed the study and coordinated the work. All authors contributed to the final version of the paper.

Published online at http://www.nature.com/naturegenetics/
Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions /

1. Lebwohl, M. Psoriasis. *Lancet* **361**, 1197–1204 (2003).
2. Jackson, B. *et al.* Late cornified envelope family in differentiating epithelia–response to calcium and ultraviolet irradiation. *J. Invest. Dermatol.* **124**, 1062–1070 (2005).
3. Krueger, J.G. & Bowcock, A. Psoriasis pathophysiology: current concepts of pathogenesis. *Ann. Rheum. Dis.* **64** Suppl 2, ii30–ii36 (2005).
4. Lowes, M.A., Bowcock, A.M. & Krueger, J.G. Pathogenesis and therapy of psoriasis. *Nature* **445**, 866–873 (2007).
5. Bowcock, A.M. & Cookson, W.O. The genetics of psoriasis, psoriatic arthritis and atopic dermatitis. *Hum. Mol. Genet.* **13** (Spec. No. 1) R43–R55 (2004).
6. Russell, T.J., Schultes, L.M. & Kuban, D.J. Histocompatibility (HL-A) antigens associated with psoriasis. *N. Engl. J. Med.* **287**, 738–740 (1972).
7. Elder, J.T. PSORS1: linking genetics and immunology. *J. Invest. Dermatol.* **126**, 1205–1206 (2006).
8. Liu, Y. *et al.* A genome-wide association study of psoriasis and psoriatic arthritis identifies new disease loci. *PLoS Genet.* **4**, e1000041 (2008).
9. Nair, R.P. *et al.* Sequence and haplotype analysis supports HLA-C as the psoriasis susceptibility 1 gene. *Am. J. Hum. Genet.* **78**, 827–851 (2006).
10. Cargill, M. *et al.* A large-scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes. *Am. J. Hum. Genet.* **80**, 273–290 (2007).
11. Hollox, E.J. *et al.* Psoriasis is associated with increased beta-defensin genomic copy number. *Nat. Genet.* **40**, 23–25 (2008).
12. Redon, R. *et al.* Global variation in copy number in the human genome. *Nature* **444**, 444–454 (2006).
13. Estivill, X. & Armengol, L. Copy number variants and common disorders: filling the gaps and exploring complexity in genome-wide association studies. *PLoS Genet.* **3**, 1787–1799 (2007).
14. Capon, F. *et al.* Searching for psoriasis susceptibility genes in Italy: genome scan and evidence for a new locus on chromosome 1. *J. Invest. Dermatol.* **112**, 32–35 (1999).
15. Korbel, J.O. *et al.* Paired-end mapping reveals extensive structural variation in the human genome. *Science* **318**, 420–426 (2007).
16. Kidd, J.M. *et al.* Mapping and sequencing of structural variation from eight human genomes. *Nature* **453**, 56–64 (2008).
17. Zhao, X.P. & Elder, J.T. Positional cloning of skin-specific genes from the human epidermal differentiation complex. *Genomics* **45**, 250–258 (1997).
18. Chen, H. *et al.* Association of skin barrier genes within the PSORS4 locus is enriched in Singaporean Chinese with early-onset psoriasis. *J. Invest. Dermatol.* advance online publication, doi:10.1038/jid.2008.273 (11 September 2008).
19. Asumalahti, K. *et al.* Coding haplotype analysis supports HCR as the putative susceptibility gene for psoriasis at the MHC PSORS1 locus. *Hum. Mol. Genet.* **11**, 589–597 (2002).
20. Nair, R.P. *et al.* Polymorphisms of the IL12B and IL23R genes are associated with psoriasis. *J. Invest. Dermatol.* **128**, 1653–1661 (2008).
21. Capon, F., Semprini, S., Dallapiccola, B. & Novelli, G. Evidence for interaction between psoriasis-susceptibility loci on chromosomes 6p21 and 1q21. *Am. J. Hum. Genet.* **65**, 1798–1800 (1999).
22. McCarroll, S.A. *et al.* Deletion polymorphism upstream of IRGM associated with altered IRGM expression and Crohn's disease. *Nat. Genet.* **40**, 1107–1112 (2008).
23. Aberg, K.M. *et al.* Co-regulation and interdependence of the mammalian epidermal permeability and antimicrobial barriers. *J. Invest. Dermatol.* **128**, 917–925 (2008).
24. Gonzalez, J.R. *et al.* Maximizing association statistics over genetic models. *Genet. Epidemiol.* **32**, 246–254 (2008).
25. Cordell, H.J. *et al.* Case/pseudocontrol analysis in genetic association studies: A unified framework for detection of genotype and haplotype associations, gene-gene and gene-environment interactions, and parent-of-origin effects. *Genet. Epidemiol.* **26**, 167–185 (2004).
26. Alkemade, J.A. *et al.* SKALP/elafin is an inducible proteinase inhibitor in human epidermal keratinocytes. *J. Cell Sci.* **107**, 2335–2342 (1994).